Tetrahedron 72 (2016) 5571–5577

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Rearrangement reactions in the fluorination of D-glucopyranoside at the C-4 position by DAST

Tzung-Sheng Lin, Wei-Tse Tsai, Pi-Hui Liang *

School of Pharmacy, College of Medicine, National Taiwan University, No. 17, Xu-Zhou Road, Taipei 100, Taiwan

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form 27 June 2016
Accepted 29 June 2016
Available online 30 June 2016

Keywords:
DAST
Fluorinated carbohydrate
Ring constrain
Glycolipid

ABSTRACT

Attempts to synthesize 4-deoxy-4-fluoro-KRN-7000 (**2**), a potential immune adjuvant, by fluorination of 4-hydroxy of α-D-glucopyranoside using DAST, were not successful. Instead, the unusual 5-deoxy-5-fluoro β-L-altrofurnoside and the corresponding 4-deoxy-4-fluoro α-D-glucopyranoside with retained configuration were obtained. In the presence of polar solvents, the reaction afforded the epoxide product, suggesting the bicyclic oxiranium ion intermediate to be involved in this reaction. Compound **2** was eventually achieved by treating 4-methanesufonated glucopyranoside with fluoride ion and found to be a weak agonist for CD1d and NKT cell activation.

1. Introduction

Fluorine is the most electronegative of the elements, and its powerful electron-withdrawing properties can profoundly affect the reactivity of the molecules that contain it. For example, substitution of hydrogen or hydroxyl groups for fluorine can change the hydrophobicity of molecules.[1] Some fluorine-containing compounds in pharmaceuticals are used for disease treatment, such as fluorouracil and capecitabine.[2] The presence of a fluorine atom in carbohydrate often increases the chemical and metabolic stability of the glycosidic bond, which in turn can enhance the therapeutic value of sugar- or nucleoside-based pharmaceuticals.[3]

α-GalCer (alpha-galactosyl ceramide, also known as KRN7000, **1**, Scheme 1), a glycolipid composed of α-linked galactose and ceramide, is the most potent ligand for invariant natural killer T cells (*i*NKT cells).[4] Interaction of α-GalCer and with *i*NKT cells results in the secretion of T helper 1 (T_H1, e.g., IFN-γ) and T helper 2 (T_H2, e.g., IL-4, IL-10) cytokines.[5] Both T_H1 and T_H2 cytokines have important biological functions. T_H1 cytokines are associated with anti-infection and antitumor activity and T_H2 cytokines are correlated with immunosuppression and immunomodulation.[5] Due to the reciprocal effects of T_H1 and T_H2 cytokines,[6] compounds with biased cytokine profiles are highly desirable. α-C-GalCer, the C-glycosidic bond of which is resistance to hydrolysis, was found to be the most selective Th1-biased compound.[7] Thus, it is believed that the stability of glycosidic bond of the glycolipid strong correlates with the cytokine secretion profile.

As part of our ongoing interest in the synthesis of cytokine biased α-GalCer-related derivatives,[8] the synthesis of 4-deoxy-4-fluoro-KRN-7000 (**2**, Scheme 1) was pursued. The presence of fluorine at the 4-position of compound **2** was expected to both enhance the metabolic stability of the glycosidic bond and allow the formation of H-bonds with TCR α-chain and main chain of Phe 29α in CD1d protein pocket.[9] During the course of this work, the 4-deoxy-4-fluoro-KRN-7000 related sphinganine analog (**3**) was reported to have similar potency as KRN-7000 in the T_H2 cytokine secretion.[10] This study revealed that compound **3** cannot activate T_H1 cytokines. Compound **3** did not have 4-α-hydroxy group at ceramide portion which was also important for cytokine stimulation,[9] thus promoted us to investigate the activity of compound **2**.

The nucleophilic fluorinating agent-DAST (diethylaminosulfur trifluoride) is widely used in the preparation of deoxyfluoro sugars.[11] Fluorination with DAST usually occurs with inversion of configuration (S_N2 mechanism), and can be used to convert the 4-hydroxy group of glucoside to 4-deoxy-4-fluoro-galactoside with moderate yield.[12] Introduction of a fluorine atom to **2** was investigated by treating intermediate **4** with DAST; however, the desired product **5** was not obtained (Scheme 1). Careful examination of the reaction mixture revealed furanoside **6** to be the major by-product in 34% yield. The unexpected nature of this discovery prompted us to investigate its formation. An alternative route to **2** was also developed and is reported herein.

* Corresponding author. Tel.: +886 2 33668694; fax: +886 2 23919098; e-mail address: phliang@ntu.edu.tw (P.-H. Liang).

http://dx.doi.org/10.1016/j.tet.2016.06.075
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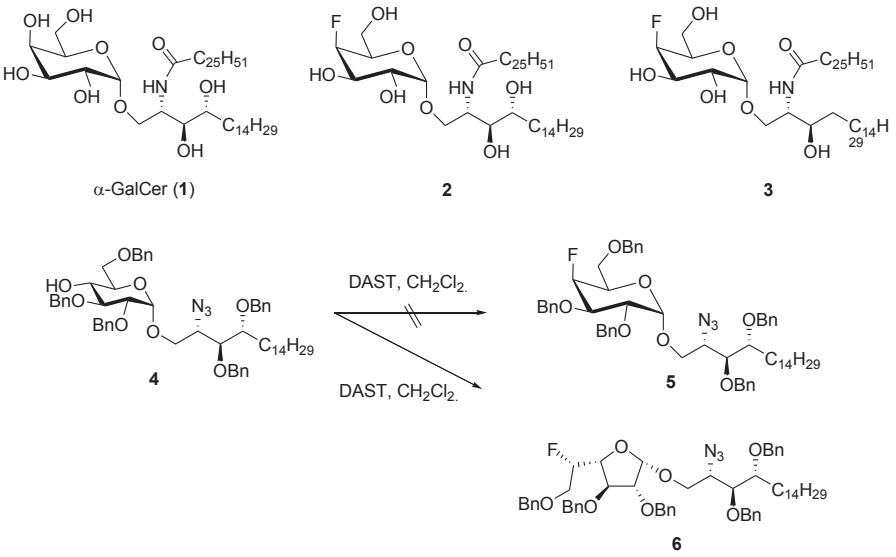


Scheme 1. Structures of α-GalCer derivatives and our attempt to synthesis of compound **2** by using DAST as a fluorinating agent.

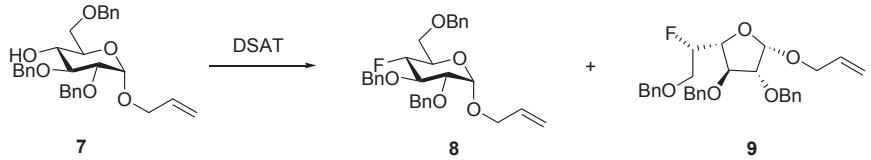
2. Results and discussion

2.1. Fluorination at the C-4 position of D-glucopyranoside using DAST

To investigate the formation of compound **6** from compound **4** (Scheme 1), allyl 2,3,6 -tri-*O*-benzyl-α-glucopyranoside (**7**)[13] was used as a model compound. To understand the scope of this reaction, it was repeated under a variety of reaction conditions (Table 1). First, compound **7** was subjected to the standard procedure, which was three-equivalents of DAST in refluxing dichloromethane (DCM) for 3 h (entry 1, Table 1). Once all the starting material had been consumed (by TLC), careful separation of products was accomplished using high performance liquid chromatography (HPLC) to give both the configuration-retained **8** and the ring-contracted furanose **9** in a ratio of 5:6 and an isolated yield of 59% (88% yield after recovery of starting material-**7**). The S_N2 type reaction to compound **7** was deduced to be of negligible significance because no configurationally inverted fluoride was detected.

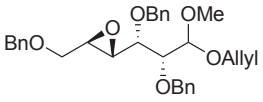
Raising the reaction temperature was found to shorten the reaction (entry 1 vs 2; and 10 vs 11, Table 1). Increasing or reducing the number of equivalents of DAST resulted in lower yields (entry 3–5), therefore, three-equivalents of DAST was conclude to be the optimized amount. Addition of either dimethylaminopyridine (DMAP), diisopropyl ethylamine (DIPEA) or tetrabutyl ammonia fluorine (TBAF) disabled the reaction (entry 6–8). Changing the solvent to diethyl ether or acetonitrile and incorporating methanol into the work-up was found to increase the ratio of furanose **9**. To our surprise, another epoxide derivative **10** (15% in diethyl ether, entry 9; 42% in acetonitrile, entry 10 and 11) was formed. Use of the anomeric β form of **7** (allyl 2,3,6-tri-*O*-benzyl β-glucopyranoside) resulted in similar outcome (entry 12).

Table 1
Results on attempted fluorination at 4-position of glucopyranose **7** in different conditions



Entry	Reagents (equiv.)	Solvent	Temp (time)	Product ratio[a]	Yield[b]
1	DAST (3)	DCM	Reflux (3 h)	5:6	59
2	DAST (3)	DCM	RT (16 h)	5:6	63
3	DAST (1)	DCM	RT (16 h)	—	NR[c]
4	DAST (5)	DCM	RT (16 h)	7:9	55
5	DAST (10)	DCM	RT (16 h)	1:1	33
6	DAST (3), DMAP (1)	DCM	RT (16 h)	—	NR
7	DAST (3), DIPEA (1)	DCM	RT (16 h)	—	NR
8	DAST (3), TBAF (1)	DCM	RT (16 h)	—	NR
9	DAST (3)	Diethyl ether	RT (16 h)	1:5	45[d]
10	DAST (3)	ACN	40 °C (3 h)	2:7	46[d]
11	DAST (3)	ACN	RT (16 h)	2:7	48[d]
12[e]	DAST (3)	DCM	Reflux (3 h)	5:6	60

[a] Ratio of **8: 9** which was determined by HPLC of reaction mixture.
[b] Isolated yields of all products being purified by HPLC.
[c] NR (no reaction, almost starting material was recovered).
[d] Additional epoxide **10** was obtained in a yield of 15–42%.
[e] Reaction of β form of **7** (allyl 2,3,6-tri-*O*-benzyl β-glucopyranoside).



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The structure of **9** was determined by NOEs experiments; the protons interactions of **9** are shown in Fig. 1 (the NOESY spectrum itself is presented in the Supplemental data). The stereochemistry at C-4 of **9** is *R* form, determined by a positive NOE interaction between H-1 and H-2; H-1 and H-4; H-2 and H-4; H-3 and H-5; and H-4 and H-5 (Fig. 1). Morishima N. et al. reported that treatment of methyl 2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranoside with DAST resulted in the formation of methyl 2,3-di-*O*-benzyl-5,6-di-deoxy-5-fluoro-β-L-altrofurnoside, in which the stereo-formation of the 5-fluoro group was verified by synthesis of its enantiomer.[14] Comparing our NMR spectrums with reported values found they were comparable, suggesting the C-5 to have the *S* configuration (Fig. 1).

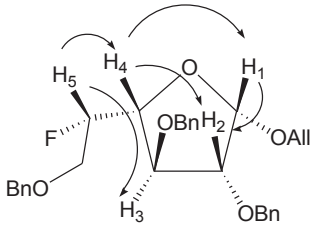

Fig. 1. Observed NOE relationships of compound **9**.

2.2. Plausible mechanism for the formation of side products

It was reported that when saccharides bearing an unprotected, somewhat hindered hydroxyl group with an electron-rich group at the *vicinal* position, are treated with DAST, a product with retained configuration and side-products showing migration of the neighboring group can be obtained.[15] In our case, the anomeric configuration of a saccharide **7** seems to be uninvolved in the formation of products (entry 1 vs 12, Table 1) and the 4-hydroxy groups in glucopyranoside **4** and **7** are not hindered. Accordingly, it is suggested that the electron-rich groups at the C-3 and C-6 positions of compounds **4** and **7** may be the contributory factors. The O-SF_2NEt_2 leaving group in compound **11** must adopt a quasi-axial orientation in a half-chair conformation of the pyranose ring (Fig. 2), resulting a disfavorable 1,3-diaxial interaction between the C-3 and C-6 positions.

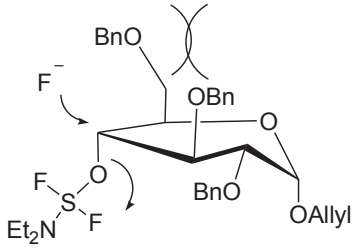

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Fig. 2. Disfavored transition state for the reaction of **7** to give fluorine inverted configuration.

Reaction of **7** with DAST in polar solvents gave stereospecific configuration-retained 4-fluorine glucopyranose **8**, 5-fluorine altrofurnose **9**, and 4,5 epoxide open-chain **10**. One plausible intermediate that accounts for the formation of these three products is compound **12** (Scheme 2). Upon formation of **11**, the 1,3-*trans* relationship between the ring oxygen and sulfonyloxyl group caused the lone pair from ring oxygen to attack the C-4 position (Scheme 2). Departure of the equatorial disposed O-SF_2NEt_2 took place with concomitant formation of the bicyclic oxiranium ion intermediate **12** which was stabilized in presence of acetonitrile (or diethyl ether). Nucleophilic attack of **12** by fluoride ion results in compound **8** (route a; attack at C-4) and **9** (route b; attack at C-5). The reaction ratio of **8** and **9** is 1:5 and 2:7 in ether and acetonitrile, respectively, indicating that the formation of **9** is a stereoelectronic effect. A lone pair of electrons located in an n-molecular orbital of nitrogen atom from acetonitrile overlaps with the antibonding σ* orbital of the O—C bond of oxirane. The delocalization of electrons favored the electron of fluorine attack at α side of **12** (route b, a kinetic route). Thus, 'route a' could be considered a themodynamic route. The reaction was monitored by TLC and quenched only once all the starting material **7** had been consumed. However, after work-up, compound **7** was again detected by TLC. For the purpose of capturing the reaction intermediate **12**, addition of a few drops of methanol in the work-up step resulted in isolation of **10**, obtained by nucleophilic attack by methanol on C-1 (route c, Scheme 2). This result is further evidence for the formation of intermediate **12** during this reaction. It is noteworthy that an analog of compound **10** has been reported as an important intermediate in the synthesis of 5-thio-L-altrose, being obtained from D-xylose in 12 steps and overall yield of ~6%.[16] In contrast, our process delivered compound **11** from D-glucose in only five steps and ~20% yield.

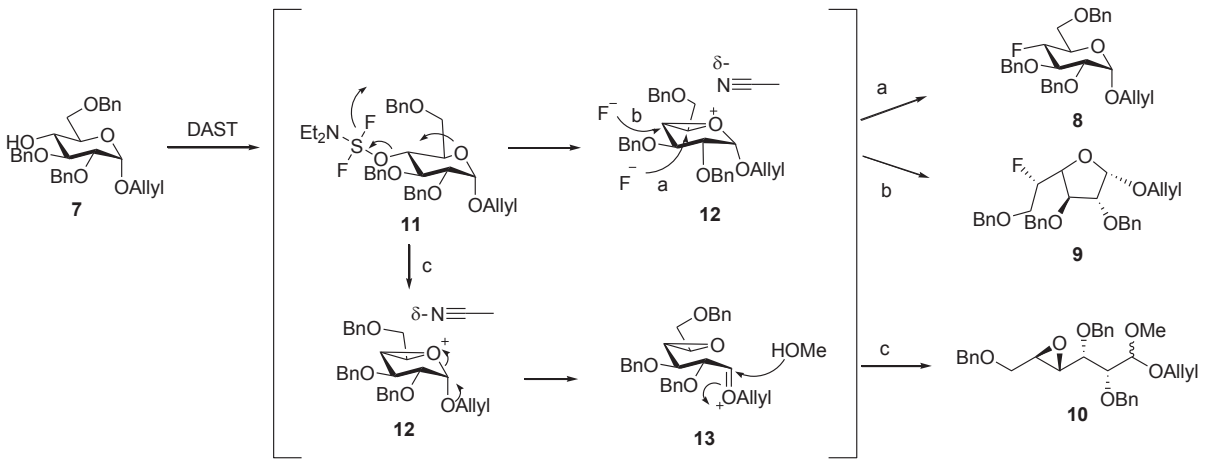
When the reaction is performed using DCM; 'route b' is presumed to dominate. There are two reasons for this assumption. First, the ratio of 5-fluoro-furanose **9** was higher than 4-fluoro-pyranose **8**. Second, there was no reaction in the presence of base (either DMAP or DIPEA), therefor S_Ni pathway is less plausible.[14]

2.3. Synthesis of compound 2

Since DAST cannot afford the configurationally inverted fluoride from **7**, installation of the axial fluoride was accomplished by a two-step process (Table 2). Treating 4-OH of glucoside with triflic anhydride (Tf_2O) and then tri(dimethylamino) sulfonium difluorotrimethylsilicate (TASF)[17] was reported to able to afford the configurationally inverted fluoride; however, in our hands, a complex mixture was obtained (entry 2, Table 2, two steps=25% yield). Use of TBAF in ACN slightly increased the reaction yield (33%, entry 4). The low yield was hypothesized to be due to the low stability of the triflic group of intermediate **14**, so the use of methanesulfonyl (Ms) group was pursued instead. Treatment of **7** with MsCl afforded 4-methanesulfonate intermediate **15**, which was attacked by a nucleophilic addition of fluoride ion from TBAF (entry 8) in ACN, gave a complete conversion of inverted fluoride in a satisfactory yield (75% for two steps). Use of DAST (entry 5) or TASF (entry 6) in place of TBAF did not result in any reaction.

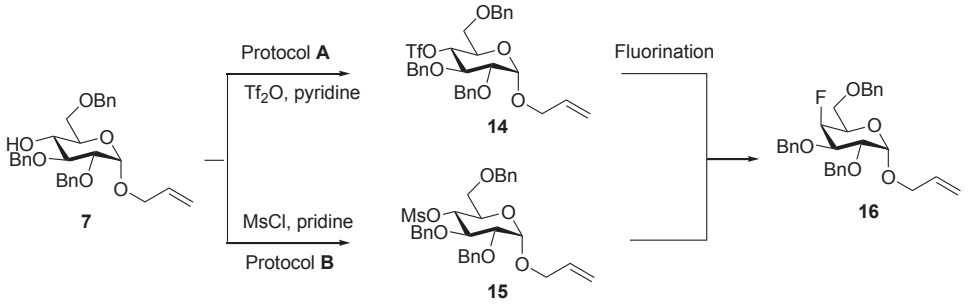
With an optimized method for the fluorination of **7** in hand, the desired compound **2** was pursued (Scheme 3). 4-*tert*-butyldimethylsilyl-2,3,6-tri-*O*-benzyl 1-thiol-D-glucopyranoside **18**[8] was activated with Me_2S_2/Tf_2O at −10 °C and then reacted with the ceramide acceptor **19**[8] to afford **20** in 54% yield. Deprotection of **20** with TBAF gave 2,3,6-tri-*O*-benzyl-D-α-glucosyl ceramide **21** in 82% yield. Treatment of **21** with methanesulfonic chloride (MsCl) afforded 4-methanesulfonate glucosyl ceramide **22**, which was attacked by TBAF to give configuration-inversed **23** in 83% yield. Debenzylation of **23** in the presence of 10% $Pd(OH)_2$ under H_2 at RT gave 4-deoxy-4-fluoro-KRN-7000 (**2**).

In order to ascertain the ability of compound **2** to activate NKT cells, a mouse B lymphoma cell line (A20-CD1d) was selected as



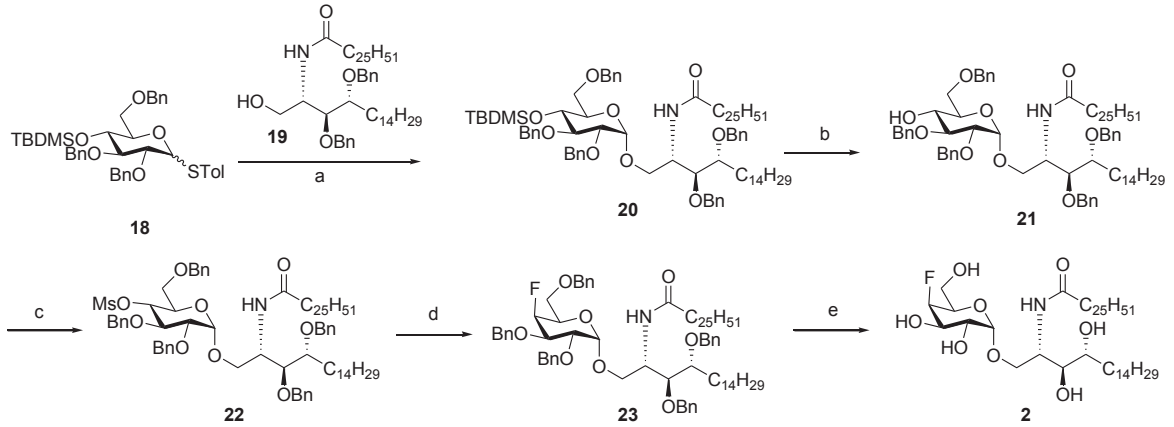
Scheme 2. Plausible mechanism for the formation of side reactions in entries 10–11 of Table 1.

Table 2
Attempts to effect the fluorination at the 4-position of glucopyranose **7** via an SN_2 mechanism



Entry	Protocol	Reagent (equiv.)	Solvent	Temp (time)	Yield[a]
1	A	DAST (3)	DCM	Reflux (16 h)	NR[b]
2	A	TASF (3)	DCM	Reflux (16 h)	25
3	A	TBAF (3)	DCM	Reflux (4 h)	23
4	A	TBAF (3)	ACN	Reflux (4 h)	33
5	B	DAST (3)	DCM	Reflux (24 h)	NR[b]
6	B	TASF (3)	DCM	Reflux (24 h)	NR[b]
7	B	TBAF (3)	DCM	Reflux (24 h)	NR[b]
8	B	TBAF (3)	ACN	Reflux (24 h)	75[b]

[a] Yield of two steps.
[b] NR (no reaction, almost starting material was recovered).



Scheme 3. Alternative route to give 4-deoxy-4fluoro-KRN-7000 (**2**). a) Me_2S_2, Tf_2O, DCM, 54%; b) TBAF, THF, 50 °C, 82%; c) MsCl, pyridine, 96%; d) TBAF, ACN, reflux, 83%; e) H_2, $Pd(OH)_2/C$, rt, 64%.

a source of antigen-presenting cells for loading with the glycolipid. After co-incubation with Vα14-TCR-expressing mNK1.2 cells as effector cells, IL-2 secretion was measured by ELISA.[8] Compound **2** induced much less IL-2 cytokine secretions than KRN-7000 (**1**), which was not found to have a significant effect (data not shown). Since α-C-GalCer was reported inactive in vitro and active in vivo,[7b] whether compound **2** is able to activate *i*NKT cells in vivo is currently being investigated, and the results will be reported in a due course.

3. Conclusion

Treatment of 4-hydroxy group of α-D-glucoside **7** with DAST gave 5-deoxy-5-fluoro β-L- altrofurnoside **9** as a major product, and the corresponding 4-deoxy-4-fluoro α-D-glucopyranoside **8** with retained configuration as the minor product. When the reaction was undertaken in the presence of polar solvents, the epoxide side product **10** was isolated, suggesting the bicyclic oxiranium ion intermediate **12** to be involved in this reaction. A novel method to synthesize compounds **9** and **10** from the relatively simple starting material- D-glucose in a few steps and with acceptable yields was developed. However, synthesis of 4-deoxy-4-fluoro-KRN-7000 (**2**) was achieved by treating 4-methanesufonated glucopyranoside with S_N2 attack of fluoride from TBAF. Though compound **2** was found to a weak agonist for CD1d and NKT cell activation, it might be of use in the immune-compromise therapy.

4. Experimental

4.1. General methods

All reagents and solvents were reagent grades and were used without further purification unless otherwise stated. The progress of the reaction was monitored by analytical TLC on 0.25 mm E. Merck silica gel 60 F_{254} using *p*-anisaldehyde, ninhydrine, and cerium molybdate as visualizing agents. Bruker-AV-400 (400 MHz) and Bruker-AV-600 (600 MHz) with TCI cryo probe and a BACS 60 sample changer. Chemical shift (δ) are given in parts per million relative to ^{1}H: 7.24 ppm, ^{13}C: 77.0 ppm for $CDCl_3$ and ^{1}H: 8.74 ppm, ^{13}C: 150.35 ppm for pyridine-D5. The splitting patterns are reported as s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet). Coupling constant (*J*) was given in Hz. Peak assignments were confirmed using 2DNMR (COSY, HSQC) techniques. Exact mass measurements were performed on VG platform electrospray ESI/MS or BioTOF II (Taiwan). Melting point was measured by a melting point apparatus (MP-1D), infrared spectroscopy was measure by FT-IR spectrometer Nicolet iS5 and optical rotation was measured by a polarimeter (Jasco DIP-370).

4.2. Biological assay

Both α-GalCer and compound **2** were dissolved in DMSO (100%) at concentrations of 1 mM. Before use, the solutions were diluted with phosphate-buffered saline (PBS, pH 7.4) to obtain final concentrations. Cytokine release from the activation of A20-CD1d system was conducted using the procedure reported previously.[8]

4.3. 2-Azido-3,4-di-*O*-benzyl-1-*O*-(5′-fluoro-2′,3′,6′-tri-*O*-benzyl-β-L-altrofurnosyl) D-*ribo*- 1,3,4-octadecan-triol (6)

To a solution of compound **4** (100 mg, 0.104 mmol, see Supplemental data for its preparation) in DCM (10 mL) was added DAST (38.4 μL, 0.31 mmol) and stirred at reflux for 3 h to afford the mixture residue. The residue was separated by column chromatography (Hexane: EtOAc, 8: 1 to 7: 1) again to get pure **6** (34 mg, 34%) as hyaline oils: $[\alpha]_D^{25}$+12 (*c* 0.0017, $CHCl_3$); IR (neat) ν_{max} 2923, 2846, 1460, 1104 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.39–7.29 (25H, m, Ph), 4.97 (1H, t, *J*=3.6 Hz, H-1), 4.67 (1H, dm, *J*=48.6 Hz, H-5), 4.74–4.51 (10H, m, CH_2Ph), 4.40 (1H, t, *J*=6.0 Hz, H-3), 4.14 (1H, m, H-4), 4.12 (1H, dd, *J*=6.0, 3.6 Hz, H-2), 4.05 (1H, dd, *J*=10.8, 2.4 Hz, H_2-7), 3.84 (1H, m, H-8), 3.67 (2H, m, H_2-6), 3.66 (1H, m, H-10), 3.65 (1H, m, H-9), 3.59 (1H, dd, *J*=10.2, 7.8 Hz, H_2-7), 1.59–1.28 (26H, m, $-CH_2CH_2-$), 0.92 (3H, t, *J*=6.6 Hz, -CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.3, 138.0, 137.9, 137.9, 137.7 (C-Ph), 128.4–127.6 (CH-Ph), 101.5 (C-1), 92.5 (d, *J*=175.5 Hz, C-5), 84.1 (C-2), 82.8 (C-3), 79.6 (C-10), 79.0 (C-9), 78.8 (d, *J*=28.5 Hz, C-4), 73.5, 73.5, 72.4, 72.4, 72.1 (CH_2Ph), 69.0 (d, *J*=19.5 Hz, C-6), 68.3 (C-7), 62.4 (C-8), 31.9–22.7 ($-CH_2CH_2$-), 14.1 ($-CH_3$); HR-ESIMS *m/z* 980.5667 $(M+Na)^+$ (calcd for $C_{59}H_{76}FN_3NaO_7$, 980.5565).

4.4. Fluorination of compound 7

4.4.1. General procedure. DAST (47.7 μL, 0.39 mmol) was added to a solution of compound **7**[13] (63.6 mg, 0.13 mmol) in either dry DCM (8 mL), dry ACN, dry diethyl ether under N_2 (Entry 1–11 of Table 1). The reaction was heated at 40 °C for 3 h or at RT for 16 h, with stirring. The mixture was diluted with a few drops of methanol and then immediately poured onto iced saturated aqueous sodium hydrogen carbonate. The aqueous layer was extracted with DCM and the combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated. The ratio for the products was monitored by the reaction residue by HPLC (C18 column (4.6 mm×250 mm, 5 μm; Thermo), MeOH: H_2O=80: 20, flow=1 mL/min, UV 214 nm). For further purification, the reaction mixture was evaporated under *vacuum* and purified by column chromatography on silica gel (Hexane: EtOAc, 9: 1) to afford the residue. The residue was purified by HPLC (syncronis C18 column (4.6 mm×250 mm, 5 μm), MeOH: H_2O=80: 20, flow=4.5 mL/min to get products **8** (retention time: 36.8), **9** (retention time 43.9 min), **10** (retention time: 28 min).

*4.4.2. Allyl 4-deoxy-4-fluoro-2,3,6-tri-O-benzyl 1-α-D-glucopyranoside (**9**).* Clear oils; $[\alpha]_D^{30}$+36 (*c* 0.04, $CHCl_3$); IR (neat) ν_{max} 3023, 1559, 1198, 1164 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.39–7.23 (15H, m, Ph), 5.93 (1H, m), 5.31 (1H, dd, *J*=17.2, 1.6 Hz), 5.21 (1H, dd, *J*=10.4, 1.6 Hz),4.84 (2H, s), 4.79 (1H, d, *J*=2.4 Hz),4.77 (1H, d, *J*=6.4 Hz), 4.63 (2H, d, *J*=12 Hz), 4.57 (2H, d, *J*=9.6 Hz), 4.52 (1H, ddd, *J*=36.8, 9.6, 8.4 Hz), 4.17 (1H, ddd, *J*=14.4, 5.2, 1.6 Hz), 4.07–3.98 (2H, m), 3.89 (1H, m), 3.67 (1H, dd, *J*=7.2, 2.4 Hz), 3.51 (1H, dd, *J*=9.6, 3.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.5, 138.0, 137.8, 133.5(CH-8), 128.4–127.5, 118.3(CH_2-9), 95.7 (C-1), 89.7 (d, *J*=182 Hz, C-4), 79.8 (d, *J*=17 Hz, C-3), 78.5, 75.2, 73.5, 73,4, 68.53 (d, *J*=18 Hz, C-5), 68.5, 68.0; HR-ESIMS *m/z* 493.2395 $(M+H)^+$ (calcd for $C_{30}H_{34}FO_5$:493.2390).

*4.4.3. Allyl 5-deoxy-5-fluoro-2,3,6-tri-O-benzyl β-L-altrofurnoside (**10**).* Hyaline oils; $[\alpha]_D^{25}$+20 (*c* 0.001, $CHCl_3$); IR (neat) ν_{max} 2924, 2873, 1105, 1027, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.33–7.25 (15H, m, Ph), 5.84 (1H, m, H-8), 5.25 (1H, dd, *J*=17.2, 1.2 Hz, H_2-9), 5.17 (1H, dd, *J*=10.8, 1.2 Hz, H_2-9), 4.87 (1H, dd, *J*=4.2, 3.6 Hz, H-1), 4.66 (1H, ddt, *J*=36.8, 6.0, 2.0, H-5), 4.63–4.54 (6H, m, CH_2Ph), 4.33 (1H, dd, *J*=6.4, 5.6 Hz, H-3), 4.10 (1H, q, *J*=2.4 Hz, H_2-7), 4.07 (1H, d, *J*=3.2 Hz, H-4), 4.05 (1H, dd, *J*=6.4, 4.2 Hz, H-2), 3.88 (1H, dd, *J*=12.8, 6.4 Hz, H_2-7), 3.78 (1H, ddd, *J*=28.4, 11.6, 2.0 Hz, H_2-6), 3.67 (1H, ddd, *J*=26.4, 11.6, 5.6 Hz, H_2-6); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.0, 137.9, 137.6 (C-Ph), 133.6 (CH-8), 128.4–127.7 (CH-Ph), 117.9 (CH_2-9), 99.7 (CH-1), 92.8 (d, *J*=175.5 Hz, CH-5), 84.2 (CH-2), 83.4 (CH-3), 78.9 (d, *J*=28.5 Hz, CH-4), 73.5, 72.6, 72.4 (CH_2Ph), 69.2 (t,

J=19.5 Hz, CH_2-6), 68.5 (CH_2-7); HR-ESIMS m/z 515.22104 $[M+Na]^+$ (calcd for $C_{30}H_{33}FO_5$ Na, 515.2210).

*4.4.4. 1-Allyl-1-methyl-2,3,6-tri-O-benzyl-4,5-epoxy-α-D-glucoside (**11**).* Hyaline oils; $[\alpha]_D^{25}$+30 (c 0.001, $CHCl_3$); IR (neat) ν_{max} 2918, 2850, 1724, 1272, 1097 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.23 (15H, m, Ph), 5.93 (1H, m), 5.29 (1H, dd, J=17.2, 1.6 Hz), 5.16 (1H, dd, J=10.4, 1.6 Hz), 4.84 (1H, d, J=11.2 Hz), 4.67–4.49 (6H, m), 4.18 (1H, dt, J=5.6, 1.6 Hz), 3.59 (1H, dd, J=6.8, 2.4 Hz), 3.52 (1H, dd, J=11.6, 2.8 Hz), 3.45 (1H, m), 3.29 (3H, s, -OCH_3), 3.24 (1H, dd, J=11.6, 6.4 Hz), 3.02 (1H, m), 3.98 (1H, dd, J=6.4, 2.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 138.3, 138.0, 134.5, 128.4–127.6, 116.9, 103.7 (CH-1), 80.1, 77.6, 77.3, 75.0, 73.3, 73.2, 70.2, 70.0, 57.0, 54.6, 54.3; HR-ESIMS m/z 505.2564 $[M+H]^+$ (calcd for $C_{31}H_{37}O_6$, 505.2585).

*4.4.5. Allyl 4-deoxy-4-fluoro-2,3,6-tri-O-benzyl 1-α-D-galactopyranoside (**16**).* The solution of **7** (49 mg, 0.1 mmol) in pyridine (3 mL) was cooled to 0 °C MsCl (10 μL) was added to the solution and stirred for 8 h. The mixture was quenched with water, and extracted with DCM for three times. The organic layers were separated and evaporated under *vacuum* to obtain compound **15** which was added ACN (5 mL) and TBAF/THF(1.0 M, 306 μL). The mixture was heated at reflux and stirred for 24 h. The mixture was quenched with $NaHCO_3$ (aq), and extracted with DCM for three times. Organic phases were separated and evaporated. The residue was purified by flash column chromatography (hexane: EtOAc, 9: 1) to get compound 16 (37 mg, 75%) as a syrup: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40–7.26 (15H, m, Ph), 5.96–5.86 (1H, m, H-8), 5.32 (1H, dd, J=17.2, 1.4 Hz, H_2-9), 5.17 (1H, dd, J=10.3, 1.2 Hz, H_2-9), 4.89 (1H, dd, J=28.3, 2.2 Hz, H-4), 4.85 (1H, d, J=3.6 Hz, H-1), 4.82–4.54 (6H, m, CH_2Ph), 4.15 (1H, dd, J=13.0, 5.1 Hz), 4.04–3.88(4H, m), 3.66 (1H, dd, J=10.8, 7.2 Hz), 3.58 (1H, dd, J=7.44, 2.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.3, 138.2, 137.8, (C-Ph), 133.6 (CH-8), 128.4–127.6 (CH-Ph), 118.1 (CH_2-9), 96.26 (CH-1), 87.5 (d, J=182 Hz, CH-4), 76.0 (d, J=18 Hz), 75.7 (CH-2), 73.6, 73.5, 72.7 (CH_2Ph), 68.5 (CH_2-7), 68.1 (d, J=18 Hz), 67.9 (d, J=6 Hz, CH-6); HR-ESIMS m/z 515.2222 $[M+Na]^+$ (calcd for $C_{30}H_{33}FO_5$ Na, 515.2210).

4.5. Synthesis of 4-deoxy-4-fluoro-KRN-7000 (2)

*4.5.1. 1-O-(4-tert-butyldimethylsilyl-2′,3′,6′-tri-O-benzyl-D-glucopyranosyl)-2-hexacosanoylamino-D-ribo-1,3,4-octadecantriol (**20**).* Compound **18**[8] (100 mg, 0.15 mmol), ceramide **19**[8] (109 mg, 0.125 mmol), and 4 Å molecular sieve (100 mg) were dried under *vacuo* for 2 h, and then DCM/THF (2/1, 6 mL) was added to the mixture. The solution was cooled to −10 °C, and then Me_2S_2-Tf_2O (1.0 M solution in DCM, 225 μL, 0.225 mmol) was added. After stirring for 30 min, the mixture was quenched with triethylamine (1 mL), diluted with DCM, filtered through Celite, and washed with DCM. The solution was extracted with water and the organic layer was separated and concentrated to get α/β mixture form which was purified by column chromatography (hexane: EtOAc, 8: 1 to 6: 1) to get pure α from **120** (96.5 mg, 54%) and its β form (48 mg, 27%) as clear oils. **20**: $[\alpha]_D^{25}$+27 (c 0.0086, $CHCl_3$); IR (neat) ν_{max} 2923, 2853, 1028, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.29–7.18 (25H, m, Ph), 6.12 (1H, d, J=8.0 Hz, NH), 5.03 (1H, d, J=8.0 Hz), 4.81–4.43 (10 H, m), 4.18 (1 H, br s), 3.99 (1H, dd, J=12.0, 4.0 Hz), 3.92 (1H, dd, J=8.0, 4.0 Hz), 3.77–3.49 (8H, m), 1.99–1.24 (74H, m, $-CH_2CH_2-$), 0.87 (6H, t, J=8.0 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 139.0, 138.7, 138.7, 137.9, 137.7, 128.4–127.1, 98.2, 81.5, 80.7, 80.1, 78.6, 74.8, 73.6, 73.4, 72.9, 72.1, 71.8, 70.8, 69.3, 69.1, 31.9–29.3, 14.1; HR-ESIMS m/z 1423.0619 $[M+H]^+$ (calcd for $C_{91}H_{144}NO_9Si$, 1423.0605).

4.5.2. 1-O-(2′,3′,6′-tri-O-benzyl-D-glucopyranosyl)-2-hexacosanoylamino-D-ribo-1,3,4-octadecantriol (21). TBAF (40.5 μL, 0.14 mmol) was subjected to the solution of **20** (80 mg, 0.056 mmol) in THF (1 mL) and warmed to 50 °C. After the mixture was stirred for 3 h at 50 °C, it was diluted with DCM and extracted three times with brine and water. The organic layers were separated, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (Hexane: EtOAc, 5: 1 to 4: 1) to afford **21** (60 mg, 82%) as a syrup; $[\alpha]_D^{25}$−15 (c 0.002, $CHCl_3$); IR (neat) ν_{max} 2923, 2853, 1455, 1057 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32–7.23 (25H, m, Ph), 6.00 (1H, d, J=8.0 Hz, NH), 4.97 (1H, d, J=12.0 Hz), 4.81–4.45 (10H, m), 4.21 (1H, br s), 3.94 (1H, dd, J=12.0, 8.0 Hz), 3.87 (1H, dd, J=8.0, 4.0 Hz), 3.80–3.72 (3H, m), 3.62–3.50 (5H, m), 2.41 (1H, s, OH), 1.98–1.23 (74H, m, $-CH_2CH_2-$), 0.87 (6H, t, J=6.0 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 138.6, 138.5, 138.0, 137.9, 137.7, 128.5–127.6, 98.4, 81.3, 79.9, 79.6, 78.9, 73.6, 73.5, 73.0, 71.8, 71.0, 70.5, 69.7, 68.8, 50.3, 31.9–29.3, 14.1; HRMS (ESI) m/z 1308.9710 $[M+H]^+$ (calcd for $C_{85}H_{130}NO_9$, 1308.9746).

*4.5.3. 1-O-(4′-fluoro-2′,3′,6′-tri-O-benzyl-D-galactopyranosyl)-2-hexacosanoylamino-D-ribo-1,3,4-octadecantriol (**23**).* The solution of **21** (60 mg, 0.046 mmol) in pyridine (2 mL) was cooled to 0 °C. Methanesulfonyl chloride (50 μL) was added to the solution and stirred for 4 h. The mixture was quenched with water, and extracted with DCM for three times. The organic layers were separated and evaporated under *vacuum* to obtain 4-*O*-methanesulfonyl-2,3,6-tri-*O*-benzyl-α-GalCer (**19**, 64 mg, 0.044 mmol, 96%) as hyaline oils. Compound **22** (32 mg, 0.022 mmol) in dry ACN (5 mL) under N_2 was subjected to TBAF/THF (1.0 M, 66 μL, 0.108 mmol) and then heated to 80–90 °C (reflux) and stirred for 1 d. The reaction mixture was diluted with EtOAc and extracted with water. The organic phases were separated and evaporated. The residue was purified by flash column chromatography (hexane: EtOAc, 7: 1–4: 1) to afford compound **23** (24 mg, 83%) as a syrup; $[\alpha]_D^{25}$−10 (c 0.001, $CHCl_3$); IR (neat) ν_{max} 2924, 2853, 1455, 1112 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.23 (25H, m, Ph), 5.94 (1H, d, J=7.2 Hz, NH), 4.82 (1H, d, J=49.8 Hz, H-4), 4.81 (1H, s, H-1), 4.81–4.72 (4H, m, CH_2Ph), 4.63–4.43 (6H, CH_2Ph), 4.21 (1H, br s, H-8), 3.93–3.80 (6H, m, H-2, H-3, H-5, H_2-7, H-9), 3.57 (2H, ddd, J=34.8, 7.2, 6.6 Hz, H_2-6), 3.49 (1H, m, H-10), 1.92–1.22 (74H, m, -CH_2CH_2-), 0.87 (6H, t, J=6.6 Hz, -CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.8 (NHCO), 138.6, 138.5, 138.2, 138.0, 137.6 (C-Ph), 128.4–127.6 (CH-Ph), 99.0 (C-1), 87.2 (d, J=183.0 Hz, C-4), 79.9 (C-10), 78.9 (C-9), 75.9 (d, J=18.0 Hz, C-3), 75.9 (C-2), 73.8, 73.6, 73.4, 72.3, 71.8 (CH_2Ph), 68.9 (CH_2-7), 68.5 (d, J=18.0 Hz, CH-5), 68.1 (d, J=6.0 Hz, CH_2-6), 50.2 (CH-8), 30.0–29.4 ($-CH_2CH_2$-), 14.1 ($-CH_3$); HR-ESIMS m/z 1310.9716 $[M+H]^+$ (calcd. for $C_{85}H_{129}FNO_8$, 1310.9702).

*4.5.4. 1-O-(4′-4′-deoxy-fluoro-D-galactopyranosyl)-2-hexacosanoylamino-D-ribo-1,3,4-octadecantriol (**2**).* **23** (24 mg, 0.018 mmol) in a mixed solvent MeOH/DCM (4/1, 1.5 mL) was hydrogenated in the presence of 10% $Pd(OH)_2$ (2.5 mg) under H_2 at RT. After stirring overnight, the mixture was filtered through Celite, and the filtrate was concentrated in *vacuo*. The residue was purified by flash column chromatography (MeOH: DCM, 10: 1 to 9: 1) to get **2** (10 mg, 0.012 mmol, 64%) as white powders: mp 229–232 °C; $[\alpha]_D^{25}$−20 (c 0.001, MeOH); IR (neat) ν_{max} 3458, 1728, 1070, 1025 cm^{-1}; ^1H NMR (d_5-pyridine, 600 MHz) δ 8.50 (1H, d, J=8.9 Hz, NH), 5.59 (1H, d, J=3.6 Hz, H-1), 5.41 (1H, dd, J=51.0, 3.6 Hz, H-4), 5.27 (1H, m, H-8), 4.68 (1H, dd, J=10.8, 5.4 Hz, H_2-7), 4.56 (1H, dd, J=3.6, 2.4 Hz, H-2), 4.54 (1H, m, H-5), 4.48 (1H, ddd, J=30.6, 10.2, 2.4 Hz, H-3), 4.39 (1H, dd, J=10.8, 4.8 Hz, H_2-7), 4.35–4.27 (4H, m, H-9, H-10, H_2-6), 2.48–1.25 (74H, m, $-CH_2CH_2-$), 0.89 (6H, t, J=6.6 Hz, -CH_3); ^{13}C NMR (d_5-pyridine, 150 MHz) δ 173.6 (NHCO), 101.6 (C-1), 91.8 (d, J=178.5 Hz, C-4), 76.9 (C-9), 72.8 (C-10), 72.0 (d, J=18.0 Hz, C-5), 70.5 (C-2), 70.3 (d, J=18.0 Hz, C-3), 69.1 (C-7), 61.1 (CH_2-6), 51.6 (C-8), 37.1–23.2

($-CH_2CH_2-$), 14.6 ($-CH_3$); HR-ESIMS *m/z* 860.7368 $[M+H]^+$ (calcd for $C_{50}H_{99}FNO_8$, 860.7349).

Acknowledgements

This work was financially supported by the Ministry of Science and Technology (MOST 104-2320-B-002 -008 -MY3), Taiwan. We thank Dr. Jung-Tung Hung and Prof. Alice L. Yu in Institute of Stem Cell and Translational Cancer Research, Chang Gung Memorial Hospital at Linkou, Taiwan for the biological evaluations.

Supplementary data

Supplementary data (Synthesis of compound **4** and NMR spectra for new compounds are provided.) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.06.075.

References and notes

1. Lee, S. S.; Greig, I. R.; Vocadlo, D. J.; McCarter, J. D.; Patrick, B. O.; Withers, S. G. *J. Am. Chem. Soc.* **2011**, *133*, 15826.
2. (a) Wang, J.; Sánchez-Roselló, M.; Aceña, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Soloshonok, V. A.; Liu, H. *Chem. Rev.* **2014**, *114*, 2432; (b) Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359.
3. Gudmundsson, K. S.; Freeman, G. A.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **2000**, *43*, 2473.
4. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626.
5. Kronenberg, M.; Rudensky, A. *Nature* **2005**, *435*, 598.
6. Smyth, M. J.; Godfrey, D. I. *Nat. Immunol.* **2000**, *1*, 459.
7. (a) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818; (b) Schmieg, J.; Yang, G.; Franck, R. W.; Van Rooijen, N.; Tsuji, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1127; (c) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631.
8. Hsieh, M. H.; Hung, J. T.; Liw, Y. W.; Lu, Y. J.; Wong, C. H.; Yu, A. L.; Liang, P. H. *Chembiochem.* **2012**, *13*, 1689.
9. Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C.; Pellicci, D. G.; Koh, R. *Nature* **2007**, *448*, 44.
10. Raju, R.; Castillo, B. F.; Richardson, S. K.; Thakur, M.; Severins, R.; Kronenberg, M.; Howell, A. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4122.
11. Dax, K.; Albert, M.; Ortner, J.; Paul, B. J. *Carbohydr. Res.* **2000**, *327*, 47.
12. Koch, K.; Chambers, R. J. *Carbohydr. Res.* **1993**, *241*, 295.
13. Mani, N. S.; Kanakamma, P. P. *Tetrahedron Lett.* **1994**, *35*, 3629.
14. Mori, Y.; Morishima, N. *Chem. Pharm. Bull.* **1992**, *40*, 826.
15. Vera-Ayoso, Y.; Borrachero, P.; Cabrera-Escribano, F.; Carmona, A. T.; Gómez-Guillén, M. *Tetrahedron: Asymmetry* **2004**, *15*, 429.
16. Uenishi, J. I.; Ohmiya, H. *Tetrahedron* **2003**, *59*, 7011.
17. Mori, Y.; Morishima, N. *Chem. Pharm. Bull.* **1991**, *39*, 1088.